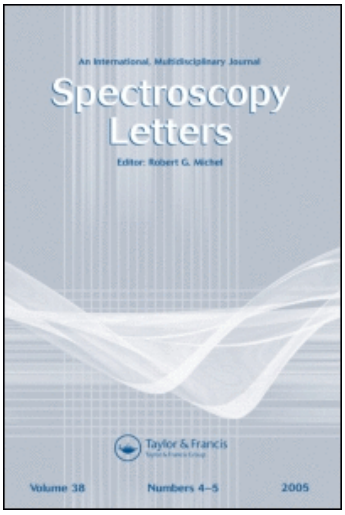

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Utility of the Least Squares Method in the PMR Spectrometric Assay of Chloramphenicol and Streptomycin with Their Degradation Products

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UTILITY OF THE LEAST SQUARES METHOD IN THE PMR SPECTROMETRIC ASSAY OF CHLORAMPHENICOL AND STREPTOMYCIN WITH THEIR DEGRADATION PRODUCTS.

Key Words: PMR spectrometry, least squares method, chloramphenicol, streptomycin, degradation products.

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ABSTRACT

The least squares line correlating Id/Is (the ratio between the signal integral values of the drug to the internal reference standard) and concentration was derived and found linear in the range of 10.0-35.0 mg chloramphenicol and 12.0-28.0 mg streptomycin. Each drug was assayed in combination with its degradation product in a ratio of 33 to 300% for the former and 33 to 200% for the latter. Commercial tablets were also assayed for the two components chloramphenicol and streptomycin existing in a ratio 1:1.

The least squares method (LSM) was statistically compared with the traditional PMR (Direct calculations) method (DCM) and official methods of assay. Illustration was made for the utility of the LSM in the determination of the molecular weight of the degraded streptomycin.

INTRODUCTION

The use of proton magnetic resonance spectrometry (PMR) in the quantitative pharmaceutical analysis is based on the direct proportionality between the integral area under a particular peak and the number of protons causing it[1-6]. Such peak areas can then be related to that of a single component of a standard solution[3] in the same solvent and under the same experimental conditions. Alternatively, this comparison may be made using another standard intety, added to the solution under test as an internal reference. The utility of the internal reference standard eliminates possible instrumental variations[7]. clearly, there should be no adverse interactions between the components in the sample and reference standards. Different internal reference standards like malonic acid[6], t-butanol[8,9], maleic acid[10,11], hexamethylcyclotrisiloxane[12] and tribromothiophene[13] have been reported to be useful in quantitative PMR procedures.

Pharmaceutical compounds existing as single and multicomponent were lately assayed using PMR technique. Examples are the determination of meprobamate in tablets[6], trimethadione in various dosage forms[9], Metformin and

chlorpropamide in tablets[14]. Assay of pharmaceutical mixtures like aspirin, phenacetin and caffeine[15] and vitamin B_1 in coexistence with vitamine B_6[16] have been also described.

In quantitative PMR spectroscopy, the peak height measurements may be useful in cases where a satisfactory separation of peaks for an accurate intergration of peak areas cannot be achieved[7].

In the present work chloramphenicol and streptomycin were selected to illustrate the utility of the least squares method in the PMR spectrometric assay of antibiotics and their degradation products in combination. More important, the quantitative PMR technique was used to determine the molecular weight of the streptomycin degradation product.

The similarity of UV spectra of chloramphenicol and its acid-induced degradation product invalidates the use of spectrophotometric method in their assay. On the other hand, the absence of a prominent maximum in the streptomycin UV spetrum makes such method not applicable for its assay.

EXPERIMENTAL

Apparatus

EM-360L NMR Varian 60 MHz spectrometer was used throughout this study.

Materials and reagents

(1) Maleic acid Analar (Hopkin and Williams Co., England).

(2) Sodium succinate (prepared from succinic acid Analar "Merck" and sodium hydroxide Analar).

(3) Sodium 3-(trimethyl-silyl) propane-1-sulfonate; DSS; (Fluka AG., Switzerland).

(4) Deuterium oxide, D_2O (Aldrich, England) and trifluoracetic acid, CF_3COOH (Fluka AG., Switzerland).

(5) Authentic chloramphenicol and its acid-induced degradation product [1-(4-nitrophenyl)-2-aminopropane-1,3-diol] were supplied by Alexandria Co., Egypt .

(6) Cidocetine vials (Cid Co., Egypt); Each vial contains chloramphenicol sodium succinate 1.378 g equivalent to 1.0 g chloramphenicol.

(7) Streptomycin sulphate vials (Cid Co., Egypt) One dose vial contains the equivalent of 1.0 g pure streptomycin base.

(8) Cidocetin capsules (Cid Co., Egypt) each capsule contains 250 mg chloramphenicol.

(9) Streptocetine capsules (Cid Co., Egypt) each capsule contains 125 mg chloramphenicol and 125 mg streptomycin sulphate.

(A) Chloramphenicol Assay

(I) Construction of Calibration Graph for Chloramphenicol:

Place weighed aliquots of chloramphenicol sodium succinate (in the range of 10-35 mg) in small clean separate glass vials. Add 0.6 ml of deuterium oxide,

containing 10 mg of the internal reference standard (sodium-3-[trimethyl-silyl] propane 1-sulfonate; DSS). Mix well till dissolve,then transfer quantitatively 0.5 ml of the clear solution to an analytical PMR tube and record the spectrum. Refer all peak field positions to DSS at 0.00 ppm. Measure the integral areas of the sharp singlets at 6.29 ppm (chloramphenicol; Id), 2.5 ppm (sodium succinate; Is) and 0.00 ppm (DSS; $Is^{`}$ )(Fig. 1). Draw the calibration curves correlating Id/Is or Id/$Is^{`}$-versus chloramphenicol concentration. Using the method of least squares, derive the regression equation of the line of best fit.

(II) Construction of Calibration Graph for the Acid-Induced Degradation Product of Chloramphenicol.

Place weighed aliquots of the degraded chloramphenicol [1-(4-nitrophenyl)-2-amino propane-1,3-diol]; in the range of 15-35 mg; in small separate clean glass vials. Add 20 mg sodium succinate to each vial. Complete as under construction of calibration graph for chloramphenicol starting from "Add 0.6 ml of deuterium oxide .....". Integrate the peaks of interest at 3.68 ppm (degraded chloramphenicol; $Id^{`}$ ), 2.5 ppm (sodium succinate; Is) and at 0.00 ppm (DSS, $Is^{`}$), (Fig. 2). Derive the least squares line correlating $Id^{`}$/Is or $Id^{`}$/$Is^{`}$ versus the degraded chloramphenicol concentrations.

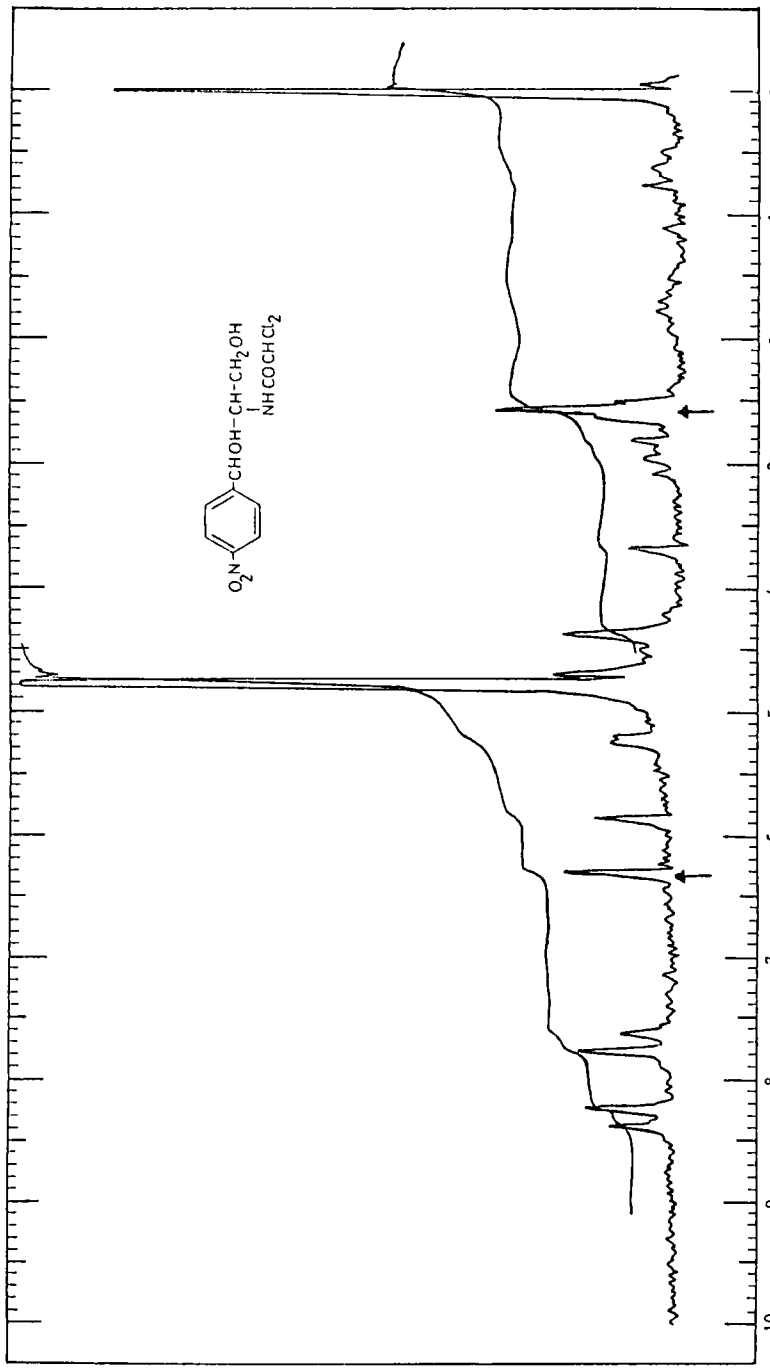

Fig. 1. PMR spectrum of 30.0 mg chloramphenicol sodium succinate in D_2O containing DSS.

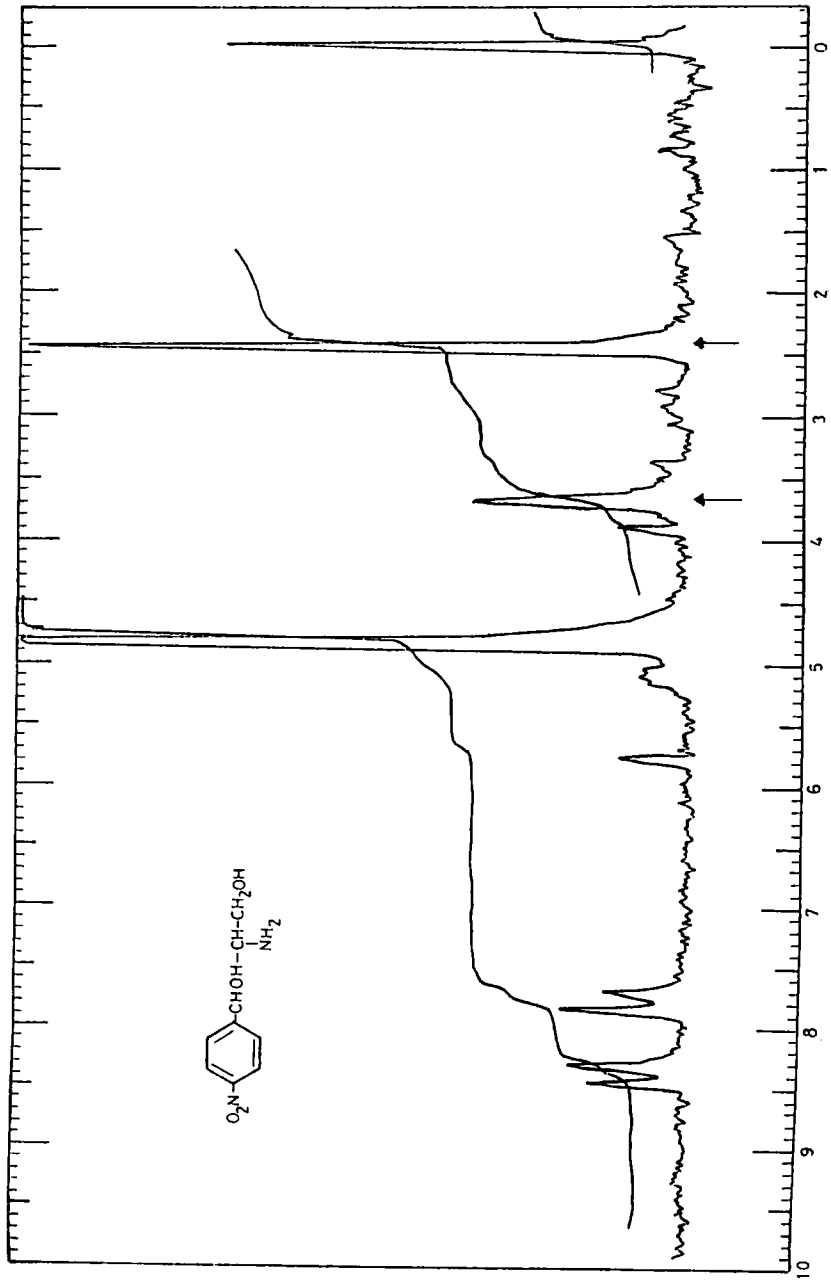

Fig. 2. PMR spectrum of 25.0 mg acid-induced degradation product of chloramphenicol in D_2O containing sodium succinate and DSS.

(III) Assay of Laboratory made mixture of chloramphenicol and its degradation product:

Weigh aliquots of chloramphenicol sodium succinate and its degradation product-in ratios cited in Table III-into clean glass vials. Add to each 20 mg sodium succinate. Complete as under calibration graph of chloramphenicol starting from "add 0.6 ml of deuterium oxide .....". Integrate the peaks of interest at 6.29 ppm (chloramphenicol; Id), 3.68 ppm (degraded chloramphenicol $\hat{Id}$) and 0.00 ppm (DSS $\hat{Is}$), (Fig. 3). Calculate the percentage recovery of the mixture components using the regression equations previously derived for the calibration lines of best fit

IV- Assay of(Cidocetin)Capsules:

In glass mortar, mix and finely powder the contents of twenty capsules. Weigh accurate portions of the fine powder (equivalent to 10-30 mg) of chloramphenicol into clean separate test tubes. Extract with two 3-ml portions of ethanol and decant through filter paper into 25-ml beakers. Evaporate the extracts to dryness and keep in vaccum desiccator for 24 hrs. Add 10 mg of DSS and 0.6 ml of trifluoroacetic acid** to each. Transfer 0.5 ml of each solution to an analytical PMR tube and record the spectrum. Refer all the peak positions to DSS at 0.00 ppm.

** Use of trifluoroacetic acid here was for the solubilization of the drug and did not alter the readings compared with standard calibration curve.

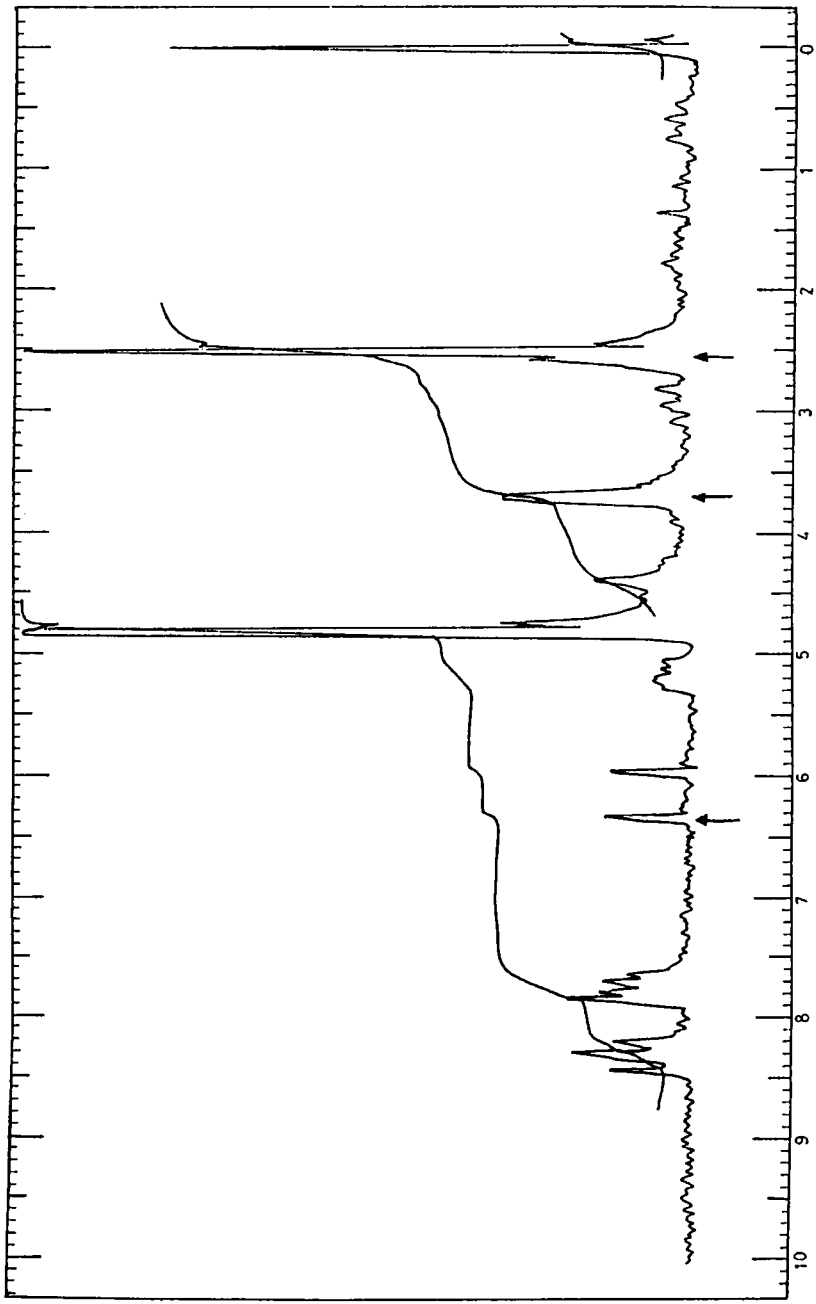

Fig. 3. PMR spectrum of mixture of 30.0 mg chloramphenicol sodium succinate and 10.0 mg of its acid-induced degradation product in D_2O containing sodium succinate and DSS.

Integrate the peaks of interest at 6.29 ppm (chloramphenicol Id) and at 0.00 ppm for DSS (Is). Calculate the percentage recovery from the corresponding regression equation.

(B) Streptomycin Assay:

(I) Construction of Calibration Graph for Streptomycin.

Place weighed quantities of streptomycin sulphate (in the range of 10-35 mg) in small clean glass vials. Add 20 mg maleic acid as internal reference standard. Then add 0.6 ml deuterium oxide containing 10 mg of DSS. Add 0.5 ml trifluoroacetic acid to induce the solubility and mix well. Transfer 0.5 ml of the clear solution to an analytical PMR tube and record the spectrum. Refer all peak field positions to DSS at 0.00 ppm. Measure the integral areas of the doublet at 1.25 ppm (streptomycin;Id) and singlet at 6.5 ppm (maleic acid;Is), (Fig.4). Draw a calibration curve correlating Id/Is versus concentration and derive the corresponding regression equation.

(II) Preparation of the Acid-Induced Degradation Product of Streptomycin:

Transfer ~ 2.0 gm streptomycin sulphate into 100-ml conical flask using 20 ml of 1N sulphuric acid. Heat in boiling water bath for 2 hrs. Concentrate the solution on a rotary evaporator maintained at 60°C until the appearance of a fine yellow precipitate. Filter by decantation

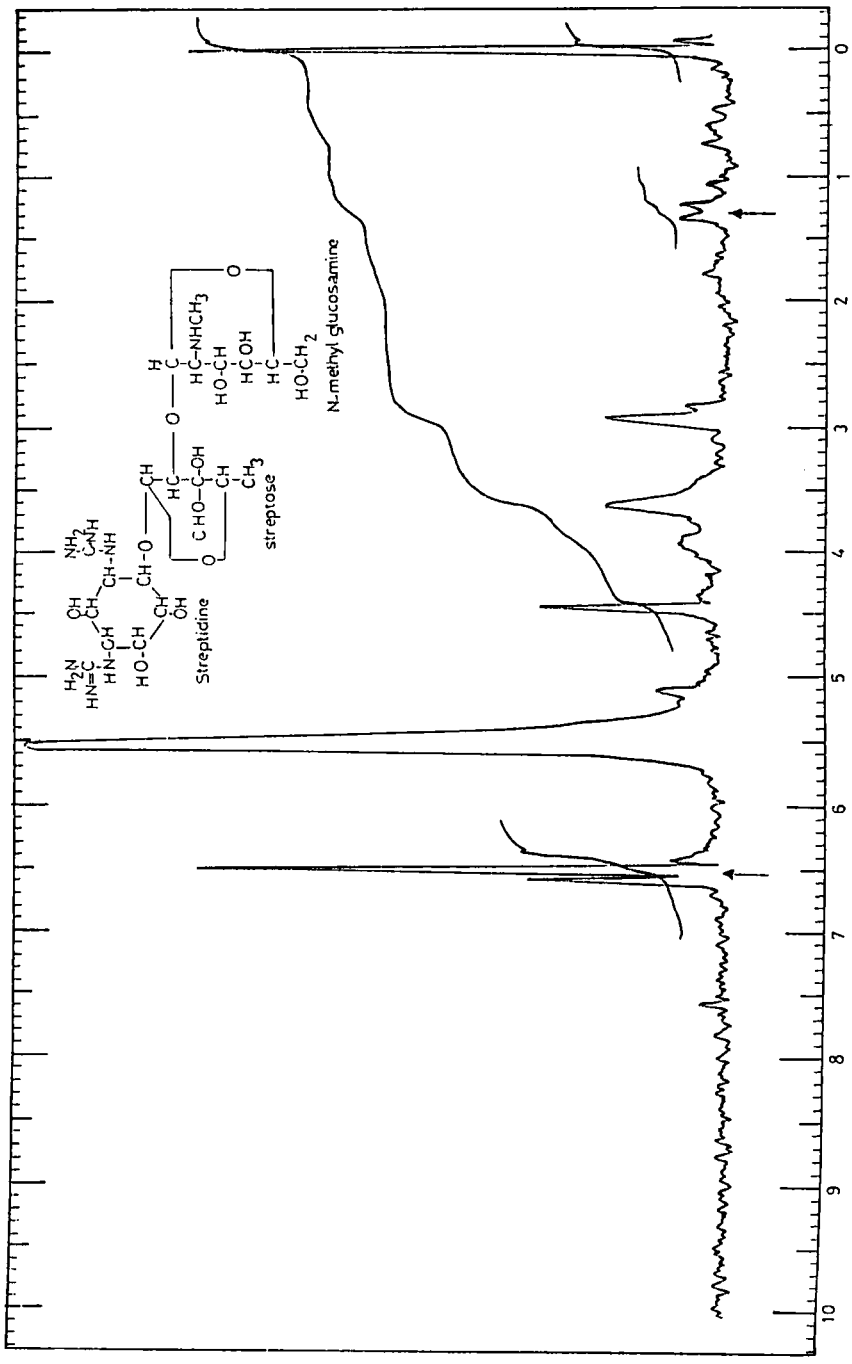

Fig. 4. PMR spectrum of 30.0 mg streptomycin sulphate in D_2O containing maleic acid and DSS.

and wash twice the residue with acetone. Dry the residue** in a vaccum desiccator.

(III) Construction of Calibration Graph for the Acid-Induced degradation Product of Streptomycin:

Place weighed aliquots of the degraded streptomycin (in the range of 15-35 mg) in small clean glass vials. Add 20.0 mg maleic acid, then add 0.6 ml deuterium oxide containing 10 mg DSS. Add 0.5 ml trifluoroacetic acid and mix well. Heat on boiling water bath for few minutes till complete solubilization. Transfer 0.5 ml of the clear solution to an analytical PMR tube and record the spectrum. Refer all peak positions to DSS at 0.00 ppm. Measure the integrals at 3.55 ppm ($I\tilde{d}$) for the acid degradation product and at 6.5 ppm (Is) for maleic acid (Fig.5). Derive the least squares line correlating $I\tilde{d}$/Is and concentration of the degraded streptomycin.

IV- Assay of Laboratory Made Mixture of Streptomycin and Its Degradation Product:

Place weighed quantities of streptomycin and its degradation products-in ratios cited in Table III-into clean glass vials. Add to each 20.0 mg maleic acid and complete as under construction of calibration graph of the

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** Following the application of TLC (silica Gel G 200 μ thick) one spot was identified using the elution system methanol: chloroform (1:9).

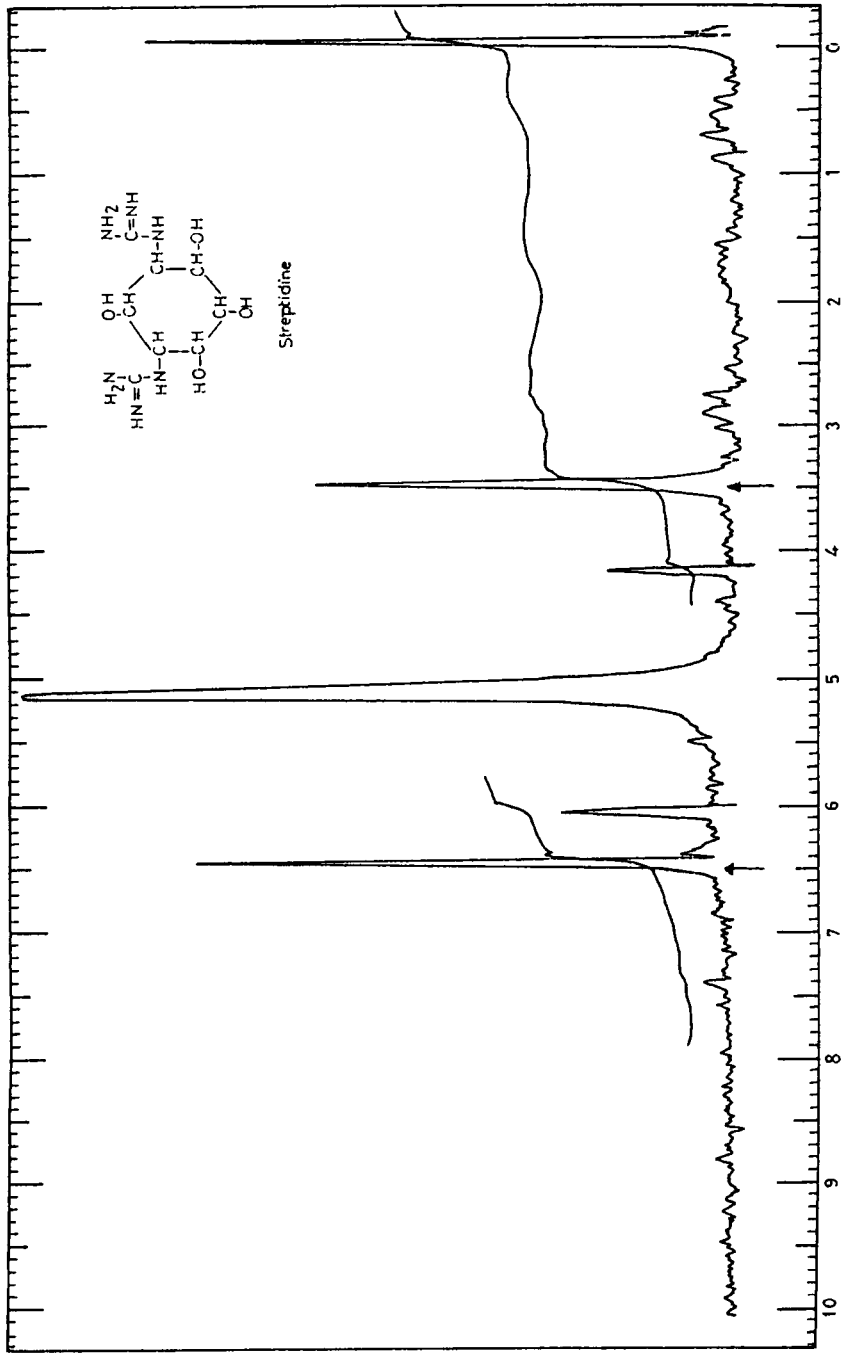

Fig. 5. PMR spectrum of 25.0 mg acid - induced degradation product of streptomycin sulphate in D_2O containing maleic and DSS.

acid-induced degradation product of streptomycin starting from "then add 0.6 ml deuterium oxide .....". Integrate the peaks of interest (Fig. 6) at 1.25 ppm (streptomycin; Id), 3.55 ppm (degraded streptomycin; $\tilde{Id}$) and 0.00 ppm (DSS; Is). Calculate the concentration of streptomycin and the degraded streptomycin from the corresponding regression equations (Table I).

V- Assay of Capsules for Chloramphenicol and Streptomycin Components (Streptocetine Capsules):

In glass mortar mix and finely powder the contents of twenty capsules. Weigh accurate portions of the fine powder equivalent to 10-30 mg of the pure drugs into clean separete test tubes. Extract with two 3-ml portions of 50% ethanol and decant through filter paper into 25-ml beakers. Evaporate the extract to dryness on a boiling water bath and keep in vaccum desiccator for 24 hrs. Complete as under construction of calibration graph for streptomycin starting from "Then add 0.6 ml deuterium oxide .....". Integrate the peaks of interest (Fig. 7), the doublet at 1.25 ppm (streptomycin) the singlet at 6.29 ppm (chloramphenicol) and the singlet at 0.00 ppm (DSS). Calculate the streptomycin and chloramphenicol concentrations using the corresponding regression equations. (Table I).

RESULTS AND DISCUSSION:

Chloramphenicol:

The PMR spectra of chloramphenicol and its acid degradation product [1-(4-nitrophenyl)-2-aminopropane-1,3-diol][17]

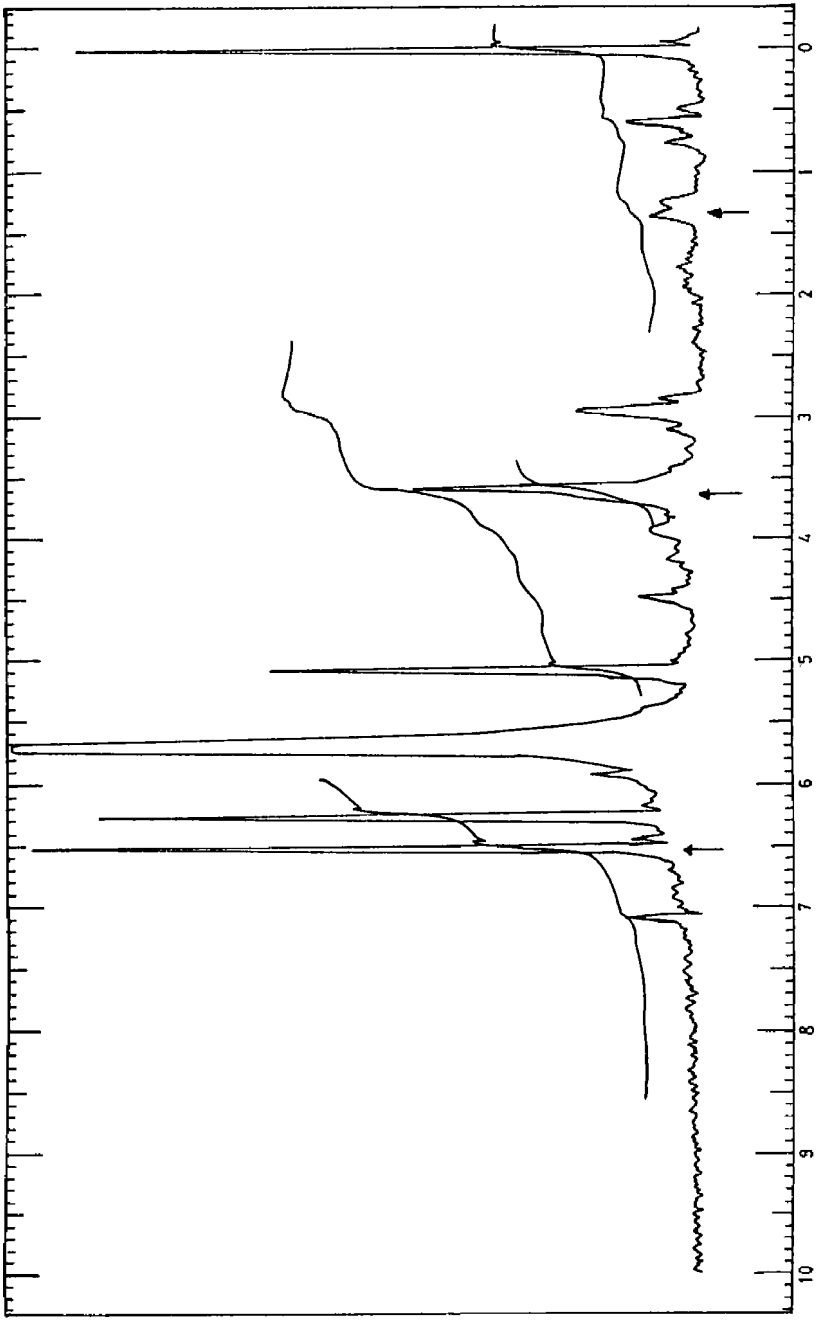

Fig. 6. PMR spectrum of mixture of 30.0 mg streptomycin sulphate and 10.0 mg of its acid - induced degradation product in D_2O containing maleic acid and DSS.

Table I: The regression data, the intercept (a), slope (b) and regression coefficient (r) for calibration curves of chloramphenicol/streptomycin and their degradation products (Internal standards: sodium succinate, maleic acid and DSS)

	$(a)X10^3$	$(b)X10^3$	(r)	$(a)X10^3$	$(b)X10^3$	(r)
Compound	Sodium succinate			DSS		
Chloramphenicol	1.305	15.185	0.9999	0.302	7.028	0.9999
Degraded-chloramphenicol	-1.800	28.720	0.9999	-3.330	32.220	0.9999
Compound	Maleic acid			DSS		
Streptomycin	-0.316	14.924	0.9999	0.988	11.638	0.9999
Degraded-streptomycin	4.739	63.406	0.9999	-	-	-

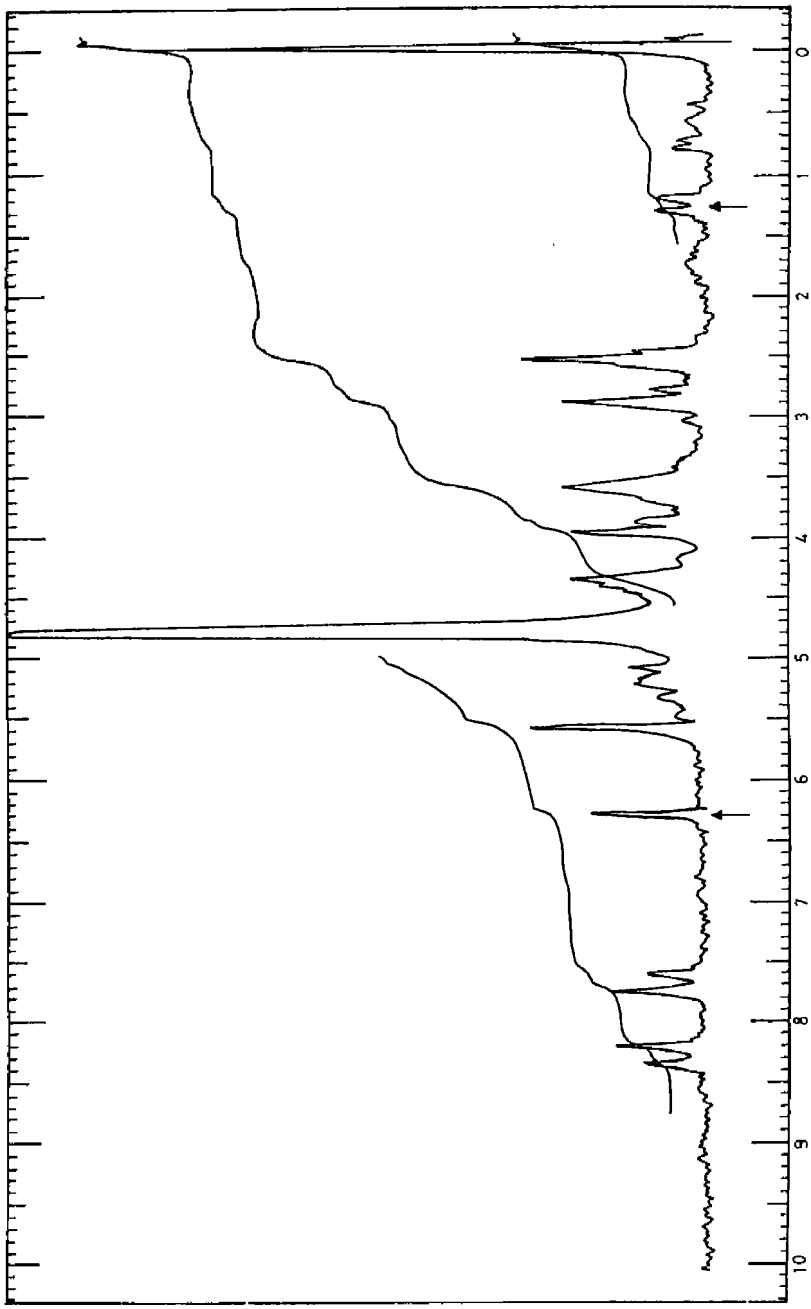

Fig. 7. PMR spectrum of mixture containing 15.0 mg chloramphenicol sodium succinate and 15.0 mg streptomycin sulphate (from capsules contents) in D_2O containing DSS.

in deuterium oxide solution are presented in Figures 1 and 2 respectively. Due to its solubilizing effect and the absence of overlaping signals in its PMR spectrum, sodium succinate was selected as internal reference standard for both compounds. All signals measured in delta scale were referred to sodium 3-(trimethyl-silyl) propane-1-sulfonate (DSS) whose singlet was positioned at 0.00 ppm. This sharp singlet (-corresponding to the nine magnetically equivalent protons-) validated the use of DSS also as internal reference standard.

The chloramphenicol spectrum is characterised by a sharp singlet at 6.29 ppm corresponding to the methine proton of the $-CHCl_2$ moiety. Such singlet is absent in the spectrum of the degraded drug (Fig. 2). The latter spectrum is characterised by the appearance of new signal at 3.68 ppm which could be integrated for the three protons of $-CH_2-OH$ and $-CH-NH_2$.

The peaks of interest at 6.29 ppm (chloramphenicol) and 3.68 ppm (degraded) were selected for their quantitative assay. These peaks were usually integrated three times with subsequent average calculations (Id & $\bar{Id}$ for chloramphenicol & its degradation product respectively). The well defined signal at 2.5 ppm exhibited by the four protons of the two equivalent methylene groups of sodium succinate were similarly integrated (Is).

The drug concentration, Wd, (direct calculations method) was estimated using the following formula[12].

$$Wd = \frac{Hs.Md}{Hd.Ms} \cdot \frac{Id}{Is} \cdot Ws \quad \text{-----} \rightarrow \text{eq.(1)}$$

where

Hs: No. of protons within the signal chosen for the internal reference standard; Hd: No. of protons within the signal chosen for the drug; Md: Molecular weight of the drug; Ms: Molecular weight of the internal reference standard; Id: Integral value of the drug signal (mm); Is: Integral value of the standard signal (mm); Ws: Weight taken of the internal reference standard. Wd: weight of the drug.

Fortunately the integration of DSS peak at 0.00 ppm may also be used as a substitute for Is in the recovery experiment calculations giving check on the assay results (Table II).

The utility of both reference standards (sodium succinate & DSS) in the chloramphenicol assay using the direct calculation method (Table II) gave results of equal accuracy (t-test) and precision (F-test). Similar assay procedure was applied to the estimation of the degraded chloramphenicol. Results of good accuracy and high precision were obtained (Table II).

Use of Least Squares method [18]:

The drug determination using the above equation (direct calculation method) necessitated the prior correct interpretation of PMR signals in order to determine precisely the Hd value (the number of protons under the selected signal).

Table II: PMR assay results of chloramphenicol, streptomycin and their acid-induced degradation product using different internal reference standards(sodium succinate, maleic acid and DSS).

	Mean* % recovery & CV%			
	LSM(i)		DCM(ii)	
compound	Sod.succinate	DSS	Sod.succinate	DSS
Chloramphenicol	99.99&0.32	100.13&0.32	99.86(a)&0.31	100.11(b)&0.28
	(0.74)	1.31	(1.34)	1.26
Degraded chloramphenicol	99.89&0.33	100.01&0.18	99.85(c)&0.32	99.89(d)&0.18
	(0.71)	3.36	(0.18)	3.17
compound	Maleic acid	DSS	Maleic acid	DSS
Streptomycin	100.02&0.29	99.98&0.14	99.56(e)&0.29	99.83(f)&0.20
	(0.28)	4.29	(1.71)	2.10
Degraded streptomycin	99.98&0.39	-	-	-

* Mean of 5-separate determinations.

- The figures in parenthesis are the calculated t-values for which the theoretical t (P=0.05) is 2.31. The underlined figures are the calculated F values for which the theoretical F (95%) is 6.39.

(ii) The mean % recovery using DCM was calculated from the formula:-

$$\% \text{ Recovery} = \left[\frac{Hs.Md.Ws}{Hd.Ms}\right] \frac{I_d}{I_s} \times \frac{100}{\text{mg taken}}$$

where the term in brackets is equal to 65.43 (a), 141.89 (b), 34.90 (c), 31.14 (d), 66.79 (e) and 85.38 (f).

LSM = Least Squares Method.

DCM = Direct Calculations Method.

Alternatively the calibration curve correlating Id/Is and drug concentration may be firstly constructed. Using the method of least squares[18] the regression equation of the line of best fit is derived.

$$Id/Is = a + b\,C \quad \longrightarrow \text{eq.(2)}$$

Where

"b" here is the slope and "a" is the intercept.

Through rearrangment of equation(1), "b" is equal to $\frac{Hd.Ms}{Hs.Md.Ws}$ providing negligible intercept.

It should be emphasized that the use of the above equation in the determination of the unknown concentration does not necessitate the knowledge of Hd nor Md; i.e. it could be also used for the assay of degradation product with unknown structure. The only criterion for such method is the linearity of the variable Id/Is as a function of drug concentration and the small intercept.

Applying the least squares method,linear relationship between Id/Is and concentration in the range of 10-35 mg (chloramphenicol) or 15-35 mg (degraded chloramphenicol) was obtained with slope (b) and intercept (a) (Table I). The regression coefficient of 0.9998 using both internal reference standards (DSS and sodium succinate) in the assay of chloramphenicol and its acid-induced degradation product indicated good linearity between the two variables "Id/Is" and "C". The applicability of the proposed method was assessed through the assay of chloramphenicol and degraded chloramphenicol as single components (Table II) and also in combination with different ratios (Table III). The results of the least squares method were compared (F & t tests) with those of the direct calculations method giving check on the analysis.

Table III: P' . assay results of two component mixtures of chloramphenicol/ streptomycin, each with its corresponding acid-induced degradation product (Internal standard: DSS).

Mixture	Mean % recovery & CV%			
	LSM		DCM	
Chloramph.: degraded chloramph. (A) : (B)	(A)	(B)	(A)	(B)
	99.80&0.58 (0.21) 1.35	100.59&0.35 (1.55) 2.53	99.72&0.50	100.27&0.22
Streptomycin: degraded strept. (C) : (D)	(C)	(D)	(C)	(D)
	100.04&0.79 (0.05) 2.31	100.12 &0.76	100.07&0.52 -	- -

- (A):(B) were taken in mg as 10:30, 20:20, 30:10 & 30:15 mg.
- (C):(D) were taken in mg as 7.97:23.92, 15.95:15.95 & 23.92:7.97 mg
- The figures in parenthesis are the calculated t-values for which the theoretical t (P=0.05) are 2.45 for chloramphenicol & 2.78 for streptomycin. The underlined figures are the calculated F values for which the theoretical F (95%) are 9.28 for chloramphenicol & 12.0 for streptomycin.
- The mean % recovery using DCM (Direct Calculation Method) was calculated as in Table II.
- LSM (Least Squares Method).

The utility of the proposed method was also appraised during the assay of pharmaceutical chloramphenicol preparation (Cidocetin capsules)(Table IV). Both the direct calculation method and the least squares method gave results of equal accuracy (t-test) & reproducibility (F-test) like those of the official method.

Table IV: PMR assay results of chloramphenicol and streptomycin in pharmaceutical preparations.

Compound / Preparation	Mean*% Recovery & CV% Chloramphenicol		
	LSM	DCM	Official method
Cidocetin capsules	99.98&0.4 (0.96) 3.61	99.89&0.41 (0.73) 3.44	99.61&0.76

Compound / Preparation	Chloramphenicol			Streptomycin		
	LSM	DCM	official	LSM	DCM	official
Streptocetin capsules	99.87&0.72 (0.54) 6.61	99.85&0.77 (0.47) 7.17	99.66&0.29	99.67&0.44 (0.71) 2.18	99.57&0.39 (1.17) 1.74	99.86&0.3

* Mean of 5 separate determination for (cidocetin) capsules & 4 separate determinations for (streptocetin) capsules.

- The figures in parenthesis are the calculated t-values for which the theoretical t(P=0.05) are 2.31 for cidocetin and 2.45 for streptocetin. The underlined figures are the calculated F-values for which the theoretical F(95%) are 6.39 for cidocetin & 9.28 for streptocetin.
- The mean % recovery using DCM (Direct calculation Method) was calculated as in Table II.
- LSM (Least Squares Method).

Streptomycin:

The PMR spectra of streptomycin and its acid-induced degradation product, each dissolved in deuterium oxide (D_2O), are presented in Figures 4 & 5. All signals were measured in delta scale and referred to DSS. The signal exhibited by the latter at 0.00 ppm could be utilized as

reference peak for quantitative measurements. Moreover maleic acid was also chosen as internal reference standard giving check on the quantitative measurements. Such choice was based on the presence of characteristic peak at 6.5 ppm due to equivalent -CH=CH- protons, where no overlaping peaks from the investigated drugs was detected. The doublet positioned at 1.25 ppm characterised the streptomycin spectrum, since it disappeared in the spectrum of the degraded streptomycin. Therefore such doublet; most probably corresponding to the 3 protons of the terminal CH_3-group of the pentose moiety, was selected for the streptomycin quantitative measurements. The signal ratios Id/Is were calculated using either DSS signal or maleic acid signal as internal reference standard (Table I).

The streptomycin recovery was computed using direct calculations method (Table II). The results using both DSS and maleic acid as internal reference standards were of equal accuracy (t-test) and precisions (F-test)(Table II). Such statistical analysis gave check on the analytical results.

The least squares method:

Calibration curves (with different internal reference standard DSS & maleic acid) correlating Id/Is versus concentration was firstly constructed. Using the method of least squares, the regression equation of the line of best fit was derived. Linear relationship with negligible intercept was obtained using concentration range of 12-28 mg

streptomycin sulphate calculated as a base. Likewise, the Id/Is values were correlated linearly with the concentration of the degraded streptomycin in a range of 12.0-28.0 mg. The regression data for the intercept (a), slope (b) and regression coefficient (r) of streptomycin and its degradation product are presented in Table I.

The advantage of the least squares method appeared here during the assay of the degraded streptomycin prior to the structure determination (c.f. direct calculation method cannot be applied in such situation).

To assess the applicability of the least squares method both streptomycin and its degradation product were assayed as single components. The assay results (Table II) using either DSS or maleic acid were of equal accuracy (t-test) and reproducibility (F-test).

Further applications of the least squares method was made to the assay of streptomycin and its degradation product mixed in different ratios (Table III) using maleic acid as internal reference standard. The former was determined through the integral value measurements at 1.25 ppm and subsequent Id/Is calculations. The recovery was computed using both direct calculation method and least squares method. For the degraded streptomycin the integral value at 3.55 ppm (Id) was measured with the subsequent calculations of the Id/Is & application of the

corresponding regression equation. Highly accurate results with good reproducibility were obtained (Table III).

Assay of Chloramphenicol and Streptomycin in Combination (Streptocetin Capsules).

Application of the proposed least squares method was here extended to the assay of the two component mixture of chloramphenicol and streptomycin existing in capsules with a ratio 1:1. For the determination of streptomycin concentration, the doublet at 1.25 ppm was chosen, while for the chloramphenicol determination, the singlet at 6.29 ppm was used (Fig. 7). The assay results were presented in Table IV. Compared with the official methods[19] (UV method for chloramphenicol and microbiological method for streptomycin), the results for both components were of equal accuracy (t-test) & equal reproducibility (F-test).

Acid Degradation of Streptomycin:

Streptomycin has been degraded using 1N sulphuric acid which cleaved the two bonds connecting the three moieties streptidine, streptose and N-Methylglucosamine with the formation of a yellow precipitate[20].

Using the method of least squares, the PMR could be utilized in determining the molecular weight of the degraded compound. For the degraded streptomycin, the calibration curve was first constructed through correlating Id/Is versus its concentration. The slope of the line of best fit was then calculated. From equations 1 and 2.

$$\text{Slope (b)} = \frac{Hd.Ms}{Hs.Md.Ws} \quad \cdots\cdots\cdots\rightarrow \quad \text{eq.(3)}$$

Rearrangements gives

$$Md = \frac{Hd.Ms}{b.Hs.Ws} \cdots\cdots\rightarrow \quad \text{eq.(4)}$$

Using certain weight of maleic acid (Ws) as internal reference standard (MW = Ms), the doublet at 6.5 ppm corresponding to -CH=CH- group could be located from its chemical shift (Hs=2). Hd could be directly obtained from the PMR spectrum providing the availability of certain functional group like the case of the acid-induced degradation product of chloramphenicol. Meanwhile, in the spectrum of acid-induced degradation product of streptomycin, the signal located at 3.55 ppm is corresponding to the cyclohexyl moiety of streptidine. Such conclusion could be made on the basis of (a) elemental analysis which indicated an imperical formula of $C_8H_{20}N_6O_8S$, obtained from the data C% 25.9, H% 5.6, N% 23.0 and S% 9.1 and (b) subtracting 14 protons for sulphate, OH, NH and NH_2 of streptidine sulphate which disappeared by deuteration. Accordingly Hd at 3.55 ppm is equal to 6 protons. Therefore, the calculation of Md according to equation (4) was equal to 274.97 corresponding to streptidine molecule (The theoretical value from the molecular formula = 262). This meant that the relative error in the determination of molecular weight using the method of least squares was equal to 4.9%.

To sum up: The least squares method could be used for the quantitative PMR analysis of antibiotics pharmaceutical preparations containing single or two components. Each component with its acid-induced degradation product in combination has been assayed with high accuracy and reprodubilibity. Such application will potentiate the utility of PMR in quantitative analysis specially for those compounds which cannot be assayed by UV spectrophotometric method because of the series spectral overlap or the absence of the appropriate chromophore. More important, the LSM was also utilized for the molecular weight determination of the acid-induced degradation product of streptomycin.

REFERENCE

1. Jackman L.M. and Sternhells S., "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry" Sec. Ed. Pergamon Press, (1969).
2. Silverstein R.M. and Bassler G.C., "Spectrometric Identification of Organic Compounds". Sec. Ed.,John Wiley and Sons, Inc., New York, London, Sydney(1969).
3. Casy A.S., "Proton Magnetic Resonance Spectroscopy". U.S. Edition, Academic Press Inc. New York (1971).
4. Kemp W., "Organic Spectroscopy" The Mcmillan Press LTD (1975).
5. El Fatatry H.M., Egypt.J.Pharm. Sci. , 56,45 (1974).
6. Turczan J.W. and Kram. T.C., J. Pharm. Sci., 56,1643 (1967).
7. Rackham D.M., Talanta, 17,895 (1970).

8. Muhtadi F.J., Hassan M.M. and Tawakkol M.M., Spectroscopy Lett., 15,373 (1982).

9. Goldwitz B. and Turczan J., J. Pharm. Sci., 62,115 (1973).

10. Tawkkol M.S., Jado A.I. and Ibrahim S.E., J. Coll. Sci., King Saudi Univ., 13, 97 (1982).

11. El Khateeb S.Z., Spectroscopy Lett., 19,193 (1986).

12. Casy A.F., Beckell A.H. and Toris A.M., Tetrahedron, 23,1405 (1967).

13. Huynh-Ngoc T. and Sirois G., J. Pharm. Sci., 62, 1334 (1973).

14. Hoda N. Assaad, M. Pharm. Thesis, Cairo, Egypt, 1987 p. 22.

15. Hollis D.P., Analyt. Chem., 35,1982 (1963).

16. Hassan S.S.M., J. Ass. Off. Anal. Chem., 61,111(1978).

17. Pietta G.P., Agnellini D., and Pace G., J. Pharm. Sci., 68, 1565 (1979).

18. Saunders L., and Fleming R.; "Mathematics and Statistics for use in the Biological and Pharmaceutical Sciences", the Pharmaceutical Press, London, Second Edition (1971) P. 199.

19- The British Pharmacopoeia, Her Majesty's stationery office-London (1980) PP. 492 and Appendix XIV A 121.

20- Wilson C.O., Gisvold O., and Doerge R.F., "Organic Medicinal and Pharmaceutical Chemistry", Eight Edition (1982) P. 261.

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